Matrilysin (PUMP) Correlates with Dermal Invasion During Appendageal Development and Cutaneous Neoplasia

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Matrix-degrading metalloproteinases play a major role in tissue remodeling. Recent studies have shown that enzymes of this class are constitutively expressed primarily by stromal cells and not by epithelium. Here we present immunohistochemical evidence that matrilysin is localized within epidermal cells in developing skin and in tumor cells of cutaneous malignancies. The expression of matrilysin protein in developing fetal skin (6–15 weeks) is localized primarily to the germinative basal cell layer of fetal epidermis and early appendageal buds. The buds continue to express matrilysin during mesenchymal invasion. As development progresses (15–19 weeks) matrilysin is concentrated only in cells at the distal portion of the invading follicular and sweat gland appendageal cords. In adult skin, matrilysin was localized specifically to the outer root sheath of the hair follicles and the secretory cells of the eccrine glands but was absent in the epidermis.

Nodulocystic, keratotic, adenoid basal cell carcinomas (BCCs) did not express matrilysin. In contrast, in the more aggressive morpheaform (infiltrative) BCCs and recurrent BCCs, matrilysin was localized at the tumor-stromal interface. In squamous cell carcinomas matrilysin was present in tumor cells at the stromal interface surrounding the tumor nests.

The demonstration of matrilysin protein in germinal basal cells during fetal skin development and its presence in tumor cells at the stromal junction suggests that this enzyme may contribute to the proteolytic activity associated with cell-extracellular matrix interactions during appendageal development and tumor invasion. ***Key words: matrix metalloproteinase/morphogenesis/cell invasion/skin tumors. J Invest Dermatol 103:482–487, 1994***

Matrix metalloproteinases (MMPs) play a major role in tissue remodeling associated with cell-stromal interactions in both physiologic and pathologic processes [1–3]. The skin is an attractive system for studying epithelial-stromal interactions during fetal morphogenesis and tumor invasion. However, little is known about how the expression of MMPs correlates with dermal invasion associated with developing epidermal appendages and whether this relates to localized tumor invasion associated with certain skin tumors (basal cell carcinomas [BCCs] and squamous cell carcinomas [SCCs]).

In previous studies [4] we have shown that in aggressive cutaneous tumors a significant quantity of 92-kDa type IV collagenase (gelatinase B) was deposited extracellularly both within the extracellular matrix adjacent to the tumor nests and in their basement membrane zone. This enzyme, and also several other MMPs (stromelysin and 72-kDa type IV collagenase / gelatinase A), did not play a major role in skin morphogenesis during fetal development [4]. Matrilysin (PUMP) has been found in tumor cells at the epithelial-stromal junction [5–7] whereas the related MMPs, interstitial collagenase [1,2] and stromelysin-3 [8,9], have been localized to stromal fibroblasts also at the stromal-epithelial interface, suggesting that these enzymes are involved in tumor progression [10,11].

Matrilysin (PUMP) is a unique member of the extracellular matrix (ECM) metalloproteinase gene family [12] and differs from other MMPs by the absence of the carboxyl terminal, hemopexin-like domain [13,14]. Matrilysin has a broad spectrum of substrate specificity *in vitro;* it can degrade fibronectin, laminin, elastin, proteoglycans, gelatin, and entactin [15,16]. The ability of matrilysin to degrade entactin [16], which is anchored to both laminin and type IV collagen, and possibly to also degrade type IV collagen [17], suggests that matrilysin might be involved in proteolytic degradation of the basement membrane.

The present study examines the immunocytochemical localization of matrilysin in fetal skin during epidermal morphogenesis and compares this to its presence in cutaneous tumors. Matrilysin expression by epidermal cells at the basement membrane zone junction of invading appendageal and tumor cells suggests that this enzyme may play a major role in ECM degradation during cutaneous development and tumor invasion.

MATERIALS AND METHODS

Forty specimens, including 28 BCCs (12 were nodulocystic, three keratotic, four adenoid, four morpheaform [infiltrative], and five recurrent), nine SCCs, one baso-squamous cell carcinoma (BSCC), and two benign sweat gland tumors (SGTs) were obtained by surgical excision. Five normal skin samples were obtained from healthy volunteers, 25–40 years of age, after

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Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma; SGT, sweat gland tumor.

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informed consent. Fetal skin samples originated from spontaneous abortions at gestational ages from 6 to 27 weeks. Specimens were embedded in Tissue-Tek O.C.T. 4583 compound and stored at −70°C. Serial cryostat sections (5–7 μ in thickness) were prepared as previously described [4], and incubated with an affinity-purified polyclonal antibody against matrilysin [17] (a gift from Dr. H. G. Welgus, Washington University). The specificity of this antibody was confirmed by Western analysis [17]. Three-step indirect immunofluorescence was used for anti-matrilysin antibody, for which a biotinylated anti-rabbit complex was used as the second antibody, followed by incubation with avidin-biotin-fluorescein isothiocyanate-containing antibody. Incubation with non-immune rabbit immunoglobulin (Ig)G or omission of the primary antibody were used as controls. Hematoxylin-stained slides of all tumors were examined by light microscopy and graded.

RESULTS

Matrilysin is Developmentally Regulated in Fetal and Adult Skin At the earliest stage of gestation examined (6 weeks) a bright intracellular staining pattern for matrilysin was present both in peridermal and in basal stratum germinativum cells of the epidermis **(Fig 1*a*)**, but was not seen in the dermal mesenchymal cells. At 8 weeks of gestation, when fetal epidermis was beginning to stratify from a two- to a three-layered epithelium, all cell layers including the basal germinative cells showed an intense staining for matrilysin **(Fig 1*b*)**. By 10 weeks of gestation the enzyme was localized intracellularly next to the basement membrane zone, forming an apparent linear fluorescent band **(Fig 1*c*)**. In contrast, the suprabasal epidermal cell compartment (stratum intermedium) continued to show matrilysin surrounding the inner portion of the cell membrane **(Fig 1*c*)**.

At the time of appendageal bud formation (in hair follicles from the scalp at 12 and 15 weeks, in those from the trunk at 18 and 20 weeks, in eccrine glands from the sole skin at 14 and 16 weeks, and in those from trunk skin at 23 and 25 weeks), bright enzyme immunostaining was first observed in all cell layers of the appendageal buds **(Fig 1*d*)**. As appendageal buds developed and began to invade the ECM, matrilysin was concentrated in the germinal cells at the distal portion of the invading follicular and sweat gland appendageal buds immediately next to the basement membrane zone **(Fig 1*e,f*)**. In addition, the suprabasal epidermal keratinocytes of the stratum intermedium overlying the developing appendages (both hair follicle and sweat gland) showed a decrease in the intensity of staining for matrilysin.

During appendageal bud invasion, a marked change in the staining pattern for matrilysin was found in the germinal epidermal basal cells between the appendageal buds. The staining intensity of intracellular matrilysin in the germinal basal cells of the inter-appendageal epidermis was significantly decreased and now showed a punctate staining pattern for matrilysin **(Fig 1*g*)**.

As hair follicle development progressed, the epithelial cells of the outer root sheath stained positively for matrilysin. Although matrilysin was also present in the developing eccrine glands, there was no difference in the distribution of enzyme protein between the primary germ cells of follicular or sweat gland appendageal buds.

By 20 weeks, matrilysin protein was no longer found in either the basal or suprabasal cell layers of the epidermis of fetal sole and scalp skin **(Fig 1*h*)** but the enzyme was still present in basal keratinocytes of trunk epidermis **(Fig 1*i*)**. At 25 and 27 weeks of gestation developing epidermis now showed the morphologic features of the postnatal state (stratification, absence of bud formation, and keratinization). Immunostaining for matrilysin was now completely absent in epidermal keratinocytes but was present in the outer root sheath of the hair follicles **(Fig 1*j*)** and in the secretory portion of the eccrine glands **(Fig 1*j*)**. Matrilysin protein was not found in either epidermal cells or stromal cells of adult skin. Occasional matrilysin-positive epithelial cells were seen in the outer root sheath of hair follicles. In contrast, the secretory cells of all the eccrine glands stained intensely for matrilysin.

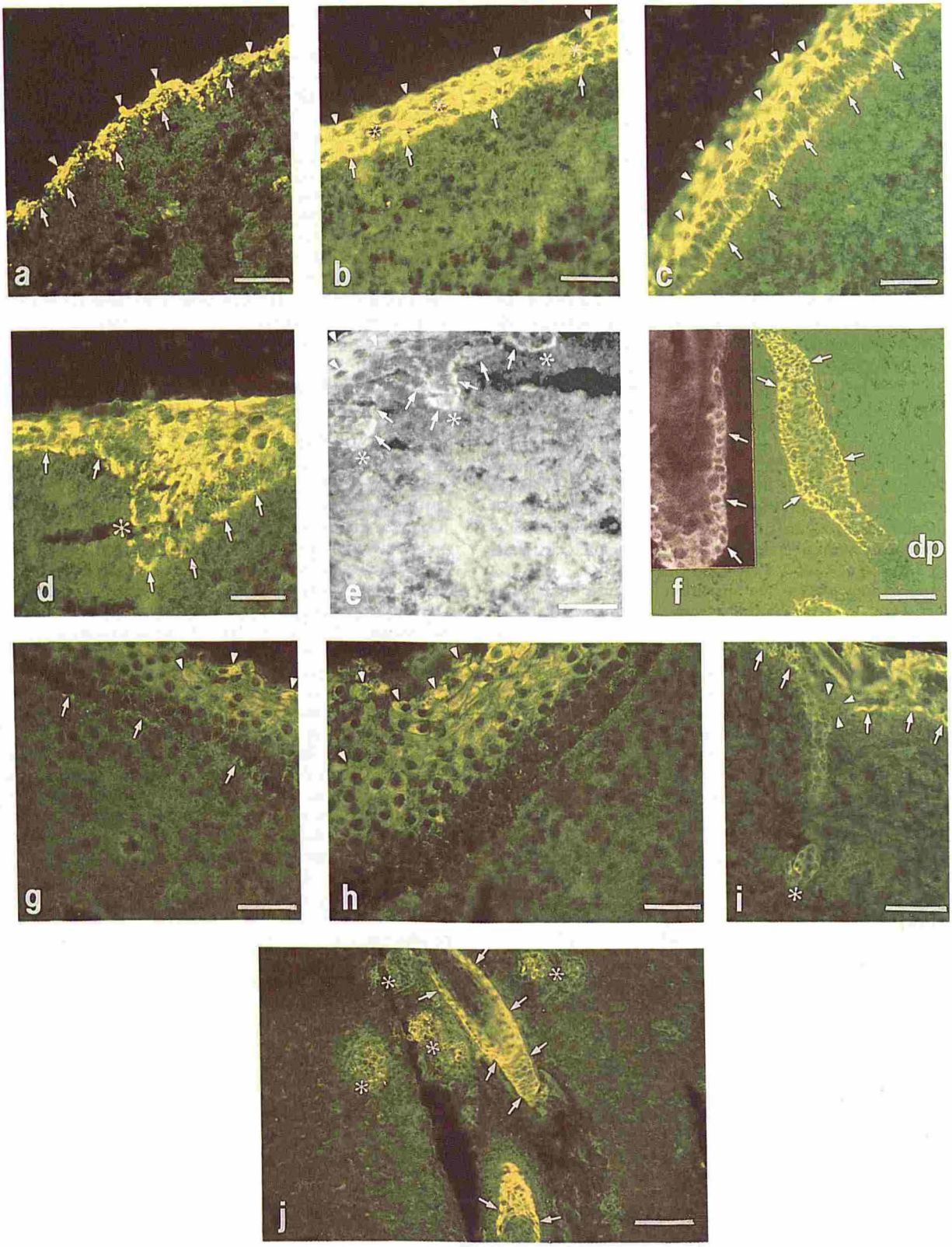
Our findings show that the presence of matrilysin protein correlates with the morphogenetic-dependent epidermal changes, suggesting that production of matrilysin is developmentally regulated.

Distribution of Matrilysin in Fetal Skin is Recapitulated in Skin Tumors To determine whether matrilysin also plays a role in the behavior of skin tumors, we compared the immunostaining pattern seen in developing fetal epidermis with the distribution of matrilysin immunoreactivity in epithelial-derived skin tumors. Interestingly, immunostaining for matrilysin was absent in the nodulocystic, keratotic, and adenoid BCCs examined. In contrast, matrilysin was present in those cancers examined considered to display more aggressive behavior [18–20], e.g., morpheaform (infiltrative) and recurrent BCCs, and SCCs. In these tumors several immunostaining patterns were identified. The most frequently observed pattern of matrilysin staining was seen in both morpheaform and recurrent BCCs. In most of these tumors the enzyme was present only in tumor cells at the tumor-stromal interface as a single cell layer forming an apparent linear band of enzyme surrounding the tumor nests **(Fig 2*a*)**. In some morpheaform and recurrent BCCs **(Fig 2*b,c*)**, a more complex immunostaining pattern for matrilysin was found. In recurrent (and morpheaform) BCCs matrilysin-positive cells were found within the tumor nests at the tumor-stromal interface, and in occasional tumor cells extending a short distance from the parent tumor nests **(Fig. 2*b,c*)**.

Of interest, the distribution of matrilysin in the epidermis adjacent to the tumor nests differed from non-tumor-associated epidermis. Matrilysin immunoreactivity was present in basal cells of the adjacent normal-appearing epidermis and occasionally was also present in the suprabasal keratinocytes.

A somewhat different pattern of matrilysin immunostaining was found in SCCs. Matrilysin-positive tumor cells were organized into small groups of cells **(Fig 2*d*)** or seen as single bright-staining tumor

Figure 1. Immunohistochemical localization of matrilysin in human fetal epidermis. *a)* Fetal skin at 6 weeks of gestation. Intense staining for matrilysin is seen in both peridermal (▶) and basal germinative cells (→). Immunoreactivity is not present in the mesenchymal cells of the ECM. *Bar,* 200 μm. *b)* Fetal skin from trunk at 8 weeks of gestation. There is bright intracellular staining of the periderm (▶), the stratum intermedium (*), and the germinative basal cells (→) of the epidermis. *Bar,* 100 μm. *c)* At 10 weeks of gestation, matrilysin is localized at the distal portion of the germinative basal cells forming an apparent linear immuno-fluorescent band next to the basement membrane zone (→). The periderm continues to stain brightly (▶). *Bar,* 50 μm. *d)* Appendageal bud (*) from fetal scalp at 15 weeks of gestation. Matrilysin is present in all epidermal cell layers of the developing appendageal buds and also shows a linear appearing immunostaining pattern along the basement membrane zone (→). *Bar,* 50 μm. *e)* Early appendageal bud invasion seen in sweat glands from the sole at 16 weeks of development. Matrilysin is concentrated predominantly in the distal portion of the cells (→) of the invading sweat gland buds (*) and is still present in the overlying periderm (▶). *Bar,* 100 μm. *f)* Hair follicle at 20 weeks of development. Matrilysin is present in germinative cells of the follicular cord next to the basement membrane zone (→) but clearly absent from the dermal papilla (dp) and the surrounding extracellular matrix. Hair follicle forms as a bulb around the specialized mesenchymal cells of the dermal papilla. *Bar,* 100 μm. Insert represents a follicle developing from an appendageal bud showing a higher power view of matrilysin containing germinative cells at the basement membrane zone (→). *Bar,* 50 μm. *g)* Inter-appendageal epidermis at the same stage shown in *e*. The staining intensity of matrilysin in the germinal basal cells of the inter-appendageal epidermis is significantly decreased and shows a punctate staining pattern (→). Cells of the periderm continue to stain brightly (*). *Bar,* 50 μm. *h)* At 20 weeks of gestation, matrilysin protein is present only in cells of the periderm (▶) of fetal sole epidermis. In contrast, in trunk skin at 20 weeks of gestation, *i)* the enzyme is still present in the basal germinative cell layer of the epidermis (→). Sweat gland appendageal cord shows a decreased intensity of staining for matrilysin in suprabasal epidermal keratinocytes (▶). The distal portion of the developing sweat gland (*) continues to stain brightly for matrilysin. *Bar,* 200 μm. *j)* Transverse section of hair follicle and sweat gland from 23-week-old fetal trunk skin. At this stage of development hair follicles and sweat glands are fully developed. The outer root sheath of the hair follicle (→) and secretory portion of the eccrine glands (*) stain brightly for matrilysin. *Bar,* 100 μm.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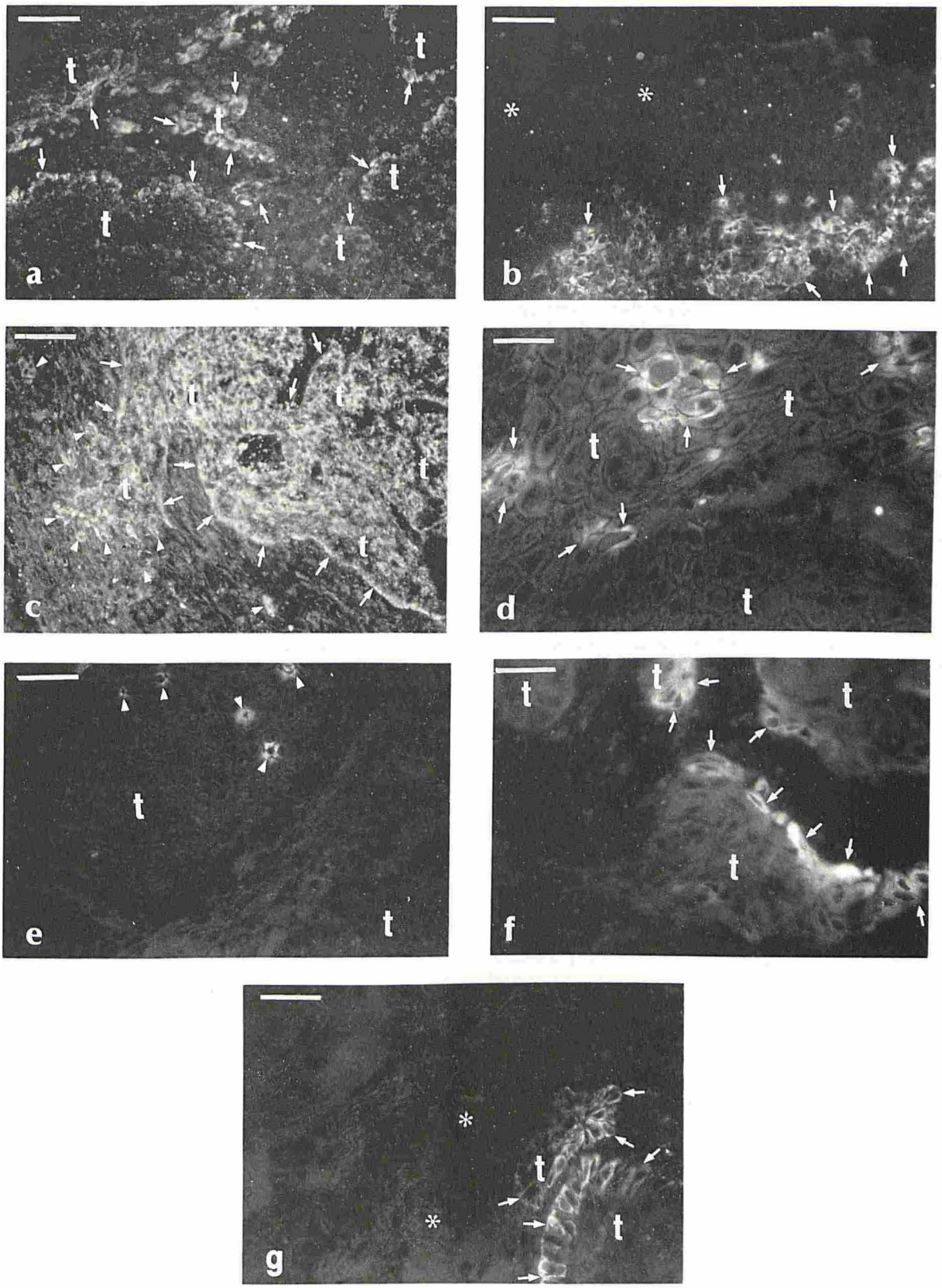

Figure 2. Distribution of matrilysin in BCCs and SCCs. *a)* Recurrent BCC. Intense immunostaining for matrilysin is seen in a single layer of tumor cells next to the tumor-stromal interface (→) and surrounding each tumor nest (t). *Bar,* 200 μm. *b)* Recurrent BCC. Matrilysin-positive cells (→) are present within the tumor nests and at the tumor-stromal interface of each developing tumor nest. Matrilysin protein is absent in the epidermal cells (*) above the tumor nests. *Bar,* 100 μm. *c)* Recurrent BCC. Matrilysin-positive cells are found within the tumor nests (t), at the tumor-stromal interface (→), and in occasional cells (▶) extending from the parent tumor nest. *Bar,* 100 μm. *d)* Well-differentiated SCC. Matrilysin-positive tumor cells are organized into small groups of cells (→) or *e)* distributed as single enzyme-containing cells (▶) among the predominantly enzyme-negative cells of the tumor nests (t). *Bar,* 50 μm. *f)* Well differentiated SCC. Matrilysin-positive tumor cells are present at the tumor-stromal interface in the tumor nests (t), (→). *Bar,* 100 μm. *g)* Poorly differentiated SCC. Matrilysin is present in tumor cells (→) around all the tumor nests (t). ECM surrounding the tumor (*) is devoid of enzyme-containing stromal cells. *Bar,* 100 μm.

cells **(Fig 2*e*)** distributed among the predominantly enzyme-negative tumor cells. In well differentiated SCCs enzyme-containing tumor cells were found infrequently at the tumor-stromal interface surrounding the tumor nests **(Fig 2*f*)**. In contrast, in poorly differentiated SCCs matrilysin was localized to tumor cells at the ECM junction around nearly all tumor nests **(Fig 2*g*)**. Matrilysin was absent in the inflammatory cells present in the ECM surrounding all tumor types examined.

Interestingly, in the two benign sweat gland tumors studied, matrilysin immunoreactivity was not detected in the tumor cells or in the stroma.

DISCUSSION

The observations presented in this study show that in developing skin, and in aggressive cutaneous tumors, matrilysin is localized in epidermal and tumor cells. This indicates that the expression of matrilysin differs markedly from that of other MMPs that are constitutively produced primarily by stromal cells [4,8,9,21]. From the earliest stages of gestation (6 weeks), matrilysin in fetal skin was found in both peridermal and germinal basal cells (stratum germinativum) of developing epidermis. Between 8 and 10 weeks of gestation, as the epidermis begins to stratify, changes in the intracellular distribution pattern of matrilysin were observed. The suprabasal cell layers of fetal epidermis (stratum intermedium) continued to stain positive for matrilysin protein. However, in the basal cells of the stratum germinativum, the enzyme was now concentrated predominantly at the epidermal-stromal interface, forming a linear-appearing immunofluorescent band.

Appendageal bud formation begins at 12–15 weeks (scalp, sole), and at 19–24 weeks (trunk). At this time matrilysin was localized predominantly in germinal and suprabasal epidermal cells of budding hair follicles and sweat glands. As appendageal development progressed, matrilysin was restricted to a specific population of epidermal cells involved in the downward invasion of the appendageal buds into the ECM, forming epidermal cell cords. During the formation of hair follicles and sweat glands, as the epidermal cords invaded the stroma, the suprabasal epidermal keratinocytes of both the developing appendages and the inter-appendageal epidermis no longer stained for matrilysin. By 25–27 weeks of gestation, fetal epidermis was completely devoid of matrilysin immunoreactivity. This was also true for postnatal and adult epidermis.

Matrilysin expression by epidermal cells at the epidermal-dermal junction during early cutaneous development and at later gestational stages, where the enzyme is restricted to the invading appendageal cords, suggests that matrilysin may contribute to proteolytic activity required for rapid, localized, ECM turnover. The functional role of matrilysin in postnatal hair follicle root sheath and sweat glands remains to be determined.

We examined several different skin tumors for the presence of matrilysin to determine whether there was a relationship between matrilysin and tumor invasion, similar to the dermal invasion that occurs during epidermal appendageal development. Significantly, in skin tumors such as recurrent and morpheaform BCCs, and in SCCs, matrilysin protein was prominently expressed in tumor cells in close contact with the basement membrane zone. In contrast, in less aggressive tumors, e.g., in nodular, nodulocystic, and adenoid BCCs, immunostaining for matrilysin was absent.

Although the expression of matrilysin protein varied among tumors considered more aggressive (morpheaform and recurrent BCCs, and SCCs) [18–20] several immunostaining patterns were identified. In the most frequently observed pattern, matrilysin was localized in tumor cells at the stromal interface surrounding the tumor nests, showing an interesting parallel with the enzyme pattern seen in the basal germinative cells of fetal epidermis and the appendageal buds. As with fetal epidermis, it was not possible to determine, at the level of the light microscope, whether any enzyme was present extracellularly adjacent to the cell membrane. Our findings show that in developing epidermal appendages and in tumor cells, matrilysin immunoreactivity was always present in close association with the basement membrane zone; matrilysin was never seen within mesenchymal cells, endothelial cells, or monocytic and lymphatic cells of the dermis.

Another pattern of matrilysin immunoreactivity was present in SCCs and was characterized by bright staining of single tumor cells, or of a group of several tumor cells, which were distributed freely among other tumor cells devoid of matrilysin. The presence of matrilysin immunoreactivity in a few selected tumor cells localized within the tumor nests suggests that transient expression of matrilysin occurs, which may reflect its normal transitory function in response to changes in cell differentiation [22].

Matrilysin has also been localized to tumor cells of gastric, colon [5,6], and prostatic [7] carcinomas, and in agreement with our observations, was not detected in stromal cells. A correlation was present between the expression of matrilysin in gastric and colon carcinomas and lymph node metastasis. Of interest are the studies of Powell *et al* [10], who transfected matrilysin cDNA into a tumorigenic but nonmetastatic human prostate tumor cell line that does not constitutively express matrilysin. The transfected tumor cells expressing matrilysin acquired a highly invasive phenotype *in vivo* with an associated increased loss of basement membrane, suggesting that matrilysin functions in the initial invasion of tumor cells. The accumulation of matrilysin protein, as we have shown previously for 92-kDa type IV collagenase [4], correlates directly with the aggressive behavior of the skin tumors studied. However, unlike matrilysin, 92-kDa type IV collagenase [1], interstitial collagenase [23], stomelysin-1 [21], and stromelysin-3 are expressed primarily by stromal cells in close proximity to the tumor but not by tumor cells.

Our data show that matrilysin is not only a specific component of a population of germinative fetal epidermal cells but is also consistently expressed in aggressive epidermal derived skin tumors in a distribution pattern similar to that seen in developing epidermis and its appendages. Matrilysin has a broad substrate specificity that overlaps with certain other matrix metalloproteinases [13,15,16]. The demonstration of matrilysin protein in germinal basal cells during fetal skin development and the presence of matrilysin in tumor cells at the stromal junction suggests that this enzyme may contribute to the changes occurring in fetal epidermis during appendageal development, and in tumor invasion.

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